CHEST

Original Research

COPD

α_1-Antitrypsin Protease Inhibitor MZ Heterozygosity Is Associated With Airflow Obstruction in Two Large Cohorts

Inga-Cecilie Sørheim, MD; Per Bakke, MD, PhD; Amund Gulsvik, MD, PhD; Sreekumar G. Pillai, PhD; Ane Johannessen, PhD; Per I. Gaarder, MD, PhD; Edward J. Campbell, MD; Alvar Agustí, MD, PhD; Peter M. A. Calverley, MD; Claudio F. Donner, MD, FCCP; Barry J. Make, MD, FCCP; Stephen I. Rennard, MD, FCCP; Jørgen Vestbo, MD, PhD; Emiel F. M. Wouters, MD, PhD, FCCP; Peter D. Paré, MD; Robert D. Levy, MD, FCCP; Harvey O. Coxson, PhD; David A. Lomas, MD, PhD; Craig P. Hersh, MD, MPH; and Edwin K. Silverman, MD, PhD

Background: **Severe α_1-antitrypsin deficiency is a known genetic risk factor for COPD. Heterozygous (protease inhibitor [PI] MZ) individuals have moderately reduced serum levels of α_1-antitrypsin, but whether they have an increased risk of COPD is uncertain.**

Methods: **We compared PI MZ and PI MM individuals in two large populations: a case-control study from Norway (n = 1,669) and a multicenter family-based study from Europe and North America (n = 2,707). We sought to determine whether PI MZ was associated with the specific COPD-related phenotypes of lung function and quantitative CT scan measurements of emphysema and airway disease.**

Results: **PI MZ was associated with a 3.5% lower FEV_1/FVC ratio in the case-control study (*P* = .035) and 3.9% lower FEV_1/vital capacity (VC) ratio in the family study (*P* = .009). In the case-control study, PI MZ also was associated with 3.7% more emphysema on quantitative analysis of chest CT scans (*P* = .003). The emphysema result was not replicated in the family study. PI MZ was not associated with airway wall thickness or COPD status in either population. Among subjects with low smoking exposure (< 20 pack-years), PI MZ individuals had more severe emphysema on chest CT scan than PI MM individuals in both studies.**

Conclusions: **Compared with PI MM individuals, PI MZ heterozygotes had lower FEV_1/(F)VC ratio in two independent studies. Our results suggest that PI MZ individuals may be slightly more susceptible to the development of airflow obstruction than PI MM individuals.**

CHEST 2010; 138(5):1125–1132

Abbreviations: AAT = α_1-antitrypsin; BD = bronchodilator; FEV_1/(F)VC = FEV_1/FVC or FEV_1/vital capacity ratios; HU = Hounsfield units; ICGN = International COPD Genetics Network; %LAA950 = percent low attenuation areas < -950 Hounsfield units; PI = protease inhibitor; SRWA-Pi10 = square root of wall area at internal perimeter of 10 mm; VC = vital capacity

Severe α_1-antitrypsin (AAT) deficiency is a known genetic risk factor for COPD,[1] but severe deficiency only accounts for about 1% to 2% of all COPD cases.[2] AAT is a protease inhibitor (PI) encoded by the SERPINA1 locus on chromosome 14q32.1. The main function of AAT is to protect the lung tissue against proteolytic stress, primarily by inactivation of the enzyme neutrophil elastase.[3] More than 100 alleles at the PI locus have been described, most of them quite rare. The most common alleles are the normal M allele (> 95% frequency worldwide) and the deficient variants S (2%-3%) and Z (1%-2%).[4] Severe AAT-deficiency is usually caused by a PI ZZ or ZNull genotype; these individuals have a markedly increased risk of COPD, especially if they smoke cigarettes.

The serum levels of AAT in PI MZ heterozygous individuals are usually lower than in PI MM individuals,[5] but whether PI MZ individuals have an increased risk of COPD remains controversial. Increased COPD risk in this group may have public health implications because there are millions of PI MZ individuals

worldwide.[4] The PI MZ genotype is relatively common compared with the severe-deficiency genotypes, and even a small increase in risk may account for a significant number of COPD cases in the population at large.

Many studies have addressed the risk of lung function reduction and COPD in PI MZ individuals, but the results have not been consistent. A metaanalysis by Hersh and colleagues[6] examined the risk of COPD in PI MZ heterozygotes. An increased risk of COPD in PI MZ vs PI MM was found in studies of COPD status, with a combined OR of 2.3 (95% CI, 1.6-3.4). However, there was no difference in mean FEV_1 between PI MZ and PI MM individuals when combining the results from population-based studies. Many of the previous studies have been limited by small sample sizes, varying phenotype definitions, or failure to adjust for smoking.

We hypothesized that investigating two large, well-characterized populations of current and ex-smokers—a case-control study and a multicenter family-based study—would enable us to address the question of PI MZ risk in more detail. In addition to examining risk of COPD, we sought to determine whether PI MZ was associated with the specific COPD-related phenotypes of lung function and quantitative CT scan measurements of emphysema and airway disease. To investigate the effect of smoking exposure level, we stratified the analysis into low- (< 20 pack-years) and high- (≥ 20 pack-years) exposure groups.

Manuscript received March 22, 2010; revision accepted May 21, 2010.

Affiliations: From the Channing Laboratory (Drs Sørheim, Hersh, and Silverman) and Pulmonary and Critical Care Division (Drs Hersh and Silverman), Brigham and Women's Hospital and Harvard Medical School, Boston, MA; Institute of Medicine (Drs Sørheim, Bakke, and Gulsvik), University of Bergen, Bergen, Norway; Department of Thoracic Medicine (Drs Bakke and Gulsvik), Haukeland University Hospital, Bergen, Norway; GlaxoSmithKline (Dr Pillai), Research Triangle Park, NC; Centre for Clinical Research (Dr Johannessen), Haukeland University Hospital, Bergen, Norway; Department of Immunology and Transfusion Medicine (Dr Gaarder), Ullevaal University Hospital, Oslo, Norway; HerediLab, Inc (Dr Campbell), Salt Lake City, UT; Institut Clinic del Torax (Dr Agustí), Hospital Clinic, Barcelona, Spain; CIBER Enfermedades Respiratorias–Fundación Caubet-Cimera (Dr Agustí), Mallorca, Spain; University of Liverpool (Dr Calverley), Liverpool, England; Division of Pulmonary Disease (Dr Donner), S. Maugeri Foundation, Veruno, Novara, Italy; National Jewish Health (Dr Make), Denver, CO; University of Nebraska (Dr Rennard), Omaha, NE; Department of Cardiology and Respiratory Medicine (Dr Vestbo), Hvidovre Hospital, Copenhagen, Denmark; North West Lung Centre (Dr Vestbo), Wythenshawe Hospital, Manchester, England; Department of Respiratory Medicine (Dr Wouters), University Hospital Maastricht, Maastricht, The Netherlands; Division of Respiratory Medicine (Dr Paré) and the James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research (Drs Paré and Coxson), University of British Columbia, St Paul's Hospital, Vancouver, BC, Canada; Department of Radiology (Drs Levy and Coxson), University of British Columbia and Vancouver General Hospital, Vancouver, BC, Canada; and Department of Medicine (Dr Lomas), University of Cambridge, Cambridge, England.
Dr Pillai is currently at Roche Pharmaceuticals (Nutley, NJ).

Funding/Support: This work was supported by the National Institutes of Health [Grants HL075478, P01 083069, R01 HL084323 (to Dr Silverman) and HL080242 (to Dr Hersh)] and a grant from the α-1 Foundation (to Dr Hersh). Both studies discussed in this article were supported by GlaxoSmithKline.

Correspondence to: Edwin K. Silverman, MD, PhD, Channing Laboratory, 181 Longwood Ave, Boston, MA 02115; e-mail: ed.silverman@channing.harvard.edu

DOI: 10.1378/chest.10-0746

Materials and Methods

Study Populations

The case-control study was performed at Haukeland University Hospital (Bergen, Norway). Case and control subjects were whites aged ≥ 40 years and current or ex-smokers of ≥ 2.5 pack-years. Case subjects had COPD with a post-bronchodilator (BD) FEV_1/FVC ratio < 0.7 and $FEV_1 < 80\%$ predicted.[7] Control subjects had normal spirometry (post-BD FEV_1/FVC, ≥ 0.7; FEV_1, $\geq 80\%$ predicted).

The family-based International COPD Genetics Network (ICGN) study included participants from 10 study centers in Europe and North America.[8,9] COPD probands were aged 45 to 65 years with a smoking history of ≥ 5 pack-years and had at least one sibling who had smoked ≥ 5 pack-years. Of the 1,723 relatives included, 51 were parents and 1,672 were siblings of their respective probands. Probands were required to have a post-BD $FEV_1 < 60\%$ predicted and FEV_1/vital capacity (VC) ratio $< 90\%$ predicted.[10] The highest value of slow VC or FVC was used as a measure of VC because FVC may underestimate VC in some subjects with COPD. This ratio, therefore, is referred to as FEV_1/VC in ICGN. $FEV_1/(F)VC$ refers to either FEV_1/FVC or FEV_1/VC ratios.

In each study population, information was collected specifically for the study. Subjects completed questionnaires on smoking history and respiratory disease as well as spirometry pre- and post-BD. In both studies, AAT phenotypes were determined by isoelectric focusing. In the present analysis, only subjects with PI type M (genotype PI MM in the vast majority of cases, and referred to as PI MM herein) or PI MZ are included. Subjects with severe AAT deficiency (eg, PI ZZ) were excluded from both studies.

Written informed consent was obtained from all participants, and the studies were approved by the respective institutional review boards (Norway case-control study, Partners Healthcare IRB Protocol Number 2009-P-000790; ICGN study, Partners Healthcare IRB Protocol Number 1999-P-011133). The Regional Committee for Medical Research Ethics (REK Vest), the Norwegian Data Inspectorate, and the Norwegian Department of Health approved the case-control study. Additional details on the study populations can be found in e-Appendix.

Chest CT Scans

High-resolution CT scans of the chest were performed in a subset of both populations. In the Norway case-control study, the subset that had chest CT scans comprised approximately the first one-half of the recruited subjects.[11] Unless a previously obtained chest CT scan was available, all probands, as well as their siblings with a > 5 pack-year smoking history, were invited for chest CT scans in the ICGN study.[8] In the Norway study, a GE LightSpeed Ultra CT scanner (Buckinghamshire, England) (1-mm slice thickness at 20-mm intervals; 120 kVp; 200 mA) was

used for all CT scans.[11] In ICGN, different CT scanners were used across centers, but the CT scanning protocol was uniform with the Norway study.[8]

Quantitative CT scan measurements of emphysema and airway dimensions have proven to be helpful in characterizing the different aspects of parenchymal and airway disease in smokers.[12] Quantitative assessment of emphysema was performed using density mask analysis with a threshold of -950 Hounsfield units (HU) to determine the percentage of lung voxels with a low attenuation area < -950 HU (%LAA950).[13] Airway wall thickness was assessed by plotting the airway lumen internal perimeter (Pi) against the square root of wall area for airways with a Pi > 6 mm and then by using this regression to predict the square root of the wall area at an internal perimeter of 10 mm (SRWA-Pi10).[14]

Statistical Analysis

Characteristics of the PI MZ and PI MM subjects were compared with *t* tests for continuous variables and χ^2 tests for categorical variables. In the case-control study, genotype-phenotype associations were assessed using linear regression models for the lung function and CT scan outcomes and logistic regression for COPD status. In the ICGN study, generalized estimating equations for categorical outcomes and random effects mixed linear models for continuous outcomes were performed to adjust for familial correlations. Family unit was included as the random effect in the models. An exchangeable correlation structure was assumed for the family members in the analysis of COPD status, and variance components covariance structure was used in the analyses of quantitative traits. Statistical analyses were performed in Stata, version 10.0 (StataCorp; College Station, TX) and SAS, version 9.1 (SAS Institute; Cary, NC).

Results

Characteristics of Study Subjects

The characteristics of study subjects in the Norway case-control study are shown in Table 1. A total of 834 cases subjects and 835 control subjects with either PI MM or PI MZ were included. Forty-four (5.3%) case subjects and 34 (4.1%) control subjects were PI MZ ($P = .244$). Quantitative emphysema data were available for 408 case subjects and 422 control subjects, and data on airway wall thickness were available for 387 cases and 393 control subjects. PI MZ control subjects had smoked more than PI MM control subjects ($P = .022$), whereas an opposite trend was noted among COPD case subjects ($P = .089$). Among case subjects, there was a trend for lower FEV_1/FVC ratio in PI MZ than in PI MM (0.49 vs 0.52; $P = .097$) (Table 1). Among control subjects, a higher FVC percent predicted was found in PI MZ than in PI MM (101.5% vs 98.0%; $P = .044$). The percentage of emphysema using a threshold of -950 HU tended to be higher in PI MZ case subjects than in PI MM case subjects (14.5% vs 10.9%; $P = .084$).

Of the 984 probands and 1,723 relatives included in the ICGN study, 43 (4.4%) and 72 (4.2%) were PI MZ, respectively. Quantitative emphysema data were available for 352 probands and 492 relatives, and data on airway wall thickness were available for 299 probands and 415 relatives. Table 2 shows the characteristics of probands and relatives by PI type. PI MZ probands tended to have a lower FEV_1/VC ratio compared with PI MM probands (0.33 vs 0.37; $P = .059$) (Table 2). Among relatives, the observed differences between PI MM and PI MZ subjects were small (not tested statistically in univariate analysis because of relatedness within the groups).

Associations Between PI Type and COPD-Related Phenotypes

We examined the associations between PI type and COPD-related phenotypes in multivariate models, adjusting for relevant covariates (Table 3). COPD was defined as Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage 2 (post-BD FEV_1/FVC, < 0.7; post-BD FEV_1, < 80% predicted) or greater in both studies. Subjects with stage 1 (only

Table 1—*Characteristics of Case and Control Subjects From the Norway Case-Control Study by Protease Inhibitor Type*

Characteristic	Case Subjects (n = 834)			Control Subjects (n = 835)		
	MM	MZ	*P* Value	MM	MZ	*P* Value
Sample, No. (%)	790 (94.7)	44 (5.3)	…	801 (95.9)	34 (4.1)	…
Male, %	60.3	65.9	ns[a]	49.7	41.2	ns
Age, y	65.4 ± 10.2	64.5 ± 9.5	ns	55.5 ± 9.6	57.8 ± 10.3	ns
Pack-y smoking	31.8 ± 18.1	27.1 ± 15.7	.089	18.7 ± 13.3	24.1 ± 10.2	.022
Post-BD spirometry						
FEV_1 % predicted	51.3 ± 17.3	48.7 ± 18.0	ns	95.1 ± 9.1	97.3 ± 8.7	ns
FVC % predicted	78.5 ± 16.4	79.6 ± 17.4	ns	98.0 ± 9.8	101.5 ± 10.1	.044
FEV_1/FVC ratio	0.52 ± 0.13	0.49 ± 0.13	.097	0.79 ± 0.04	0.78 ± 0.03	ns
CT scan, emphysema, No.	376	32	…	407	15	…
%LAA950	10.9 ± 11.2	14.5 ± 13.9	.084	1.2 ± 2.3	0.8 ± 0.9	ns
CT scan, airways, No.	357	30	…	378	15	…
SRWA-Pi10, cm	0.49 ± 0.03	0.50 ± 0.04	ns	0.48 ± 0.03	0.49 ± 0.04	ns

Data are presented as mean ± SD, unless otherwise indicated. BD = bronchodilator; ns = not significant; %LAA950 = percent low attenuation areas < -950 Hounsfield units; SRWA-Pi10 = square root of wall area at internal perimeter of 10 mm.
[a]$P \geq .10$ are not shown.

Table 2—*Characteristics of Probands and Relatives in the Multicenter ICGN Family Study by Protease Inhibitor Type*

Characteristic	Probands (n = 984)		Relatives (n = 1,723)	
	MM	MZ	MM	MZ
Sample, No. (%)	941 (95.6)	43 (4.4)	1,651 (95.8)	72 (4.2)
Male, %	60.7	51.2	50.6	52.8
Age, y	58.3 ± 5.5	57.8 ± 4.6	57.8 ± 9.4	57.5 ± 9.8
Pack-y smoking	52.5 ± 28.9	45.9 ± 22.6	39.3 ± 25.2	33.3 ± 20.9
Post-BD spirometry, No.	904	42	1,591	72
FEV_1 % predicted	35.8 ± 12.7	34.7 ± 13.3	83.6 ± 25.9	83.0 ± 27.5
FVC % predicted	73.2 ± 20.2	78.0 ± 25.6	101.1 ± 22.1	104.0 ± 20.7
FEV_1/VC ratio	0.37 ± 0.11	0.33 ± 0.12	0.64 ± 0.14	0.62 ± 0.15
CT scan, emphysema, No.	336	16	465	27
%LAA950	26.2 ± 14.9	27.0 ± 15.8	18.6 ± 11.7	22.8 ± 9.4
CT scan, airways, No.	288	11	395	20
SRWA-Pi10, cm	0.49 ± 0.04	0.47 ± 0.03	0.48 ± 0.04	0.48 ± 0.04

Data are presented as mean ± SD, unless otherwise indicated. ICGN = International COPD Genetics Network; VC = vital capacity. See Table 1 legend for expansion of other abbreviations.

present in ICGN [n = 340]) were excluded from the analysis of COPD status. In the case-control study, PI MZ heterozygotes did not have significantly higher risk of COPD than PI MM individuals (OR, 1.2; 95% CI, 0.7-2.0), after adjustment for age, sex, and pack-years. In ICGN, there was a trend for PI MZ to be associated with increased risk of COPD (OR, 1.5; 95% CI, 0.9-2.3; $P = .111$).

PI MZ was a significant predictor of lower FEV_1/FVC ratio in the multivariate analysis of all case-control subjects, after adjusting for pack-years, age, sex, and height (Table 3). PI MZ was associated with a 3.5% lower FEV_1/FVC ratio compared with PI MM ($P = .035$). This difference was still significant when adjusting for case-control status ($\beta = -0.023$; $P = .032$) and after restricting the analysis to cases only ($\beta = -0.038$; $P = .048$). This finding was confirmed in the whole ICGN cohort, using random effects mixed linear models. After adjusting for age, sex, height, pack-years, and familial correlations, PI MZ was associated with a 3.9% lower FEV_1/VC ratio compared with PI MM ($P = .009$). The results in probands only were suggestive of a similar trend, because PI MZ in probands was associated with lower FEV_1/VC ratio than PI MM ($\beta = -0.033$; $P = .066$).

In the case-control study, PI MZ was also a predictor of more emphysema (%LAA950) both in the whole group ($\beta = 3.7\%$; $P = .003$) and in cases only ($\beta = 3.8\%$; $P = .048$) after adjusting for age, sex, pack-years, and weight (Table 3). For all case and control subjects, the difference was still significant after adjusting for case-control status ($\beta = 2.5\%$; $P = .033$). The association with emphysema on CT scan was not replicated in the entire ICGN population. PI MZ was not associated with airway wall thickness (SRWA-Pi10) in either study.

Results Stratified by Level of Smoking Exposure

We stratified the study populations by level of smoking exposure (Table 4). In the Norway case-control study, there was a marked difference between results in case subjects with < 20 pack-years of smoking (low exposure) and case subjects with ≥ 20 pack-years of smoking (high exposure). PI MZ was associated with increased risk of COPD, lower FEV_1, lower FEV_1/FVC ratio, and more emphysema (%LAA950)

Table 3—*Multivariate Analysis of the Association Between PI MZ and COPD-Related Phenotypes*

Study	COPD		FEV_1, L[a]		FEV_1/(F)VC[a]		%LAA950	
	OR (95% CI)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
Case-control								
All subjects	1.2 (0.7-2.0)	ns[b]	-0.11 (0.09)	ns	-0.035 (0.02)	.035	3.7 (1.3)	.003
Case subjects only	…	…	-0.11 (0.09)	ns	-0.038 (0.02)	.048	3.8 (1.9)	.048
ICGN								
All subjects	1.5 (0.9-2.3)	ns	-0.09 (0.08)	ns	-0.039 (0.01)	.009	0.4 (1.6)	ns
Probands only	…	…	-0.039 (0.06)	ns	-0.033 (0.02)	.066	-0.2 (2.9)	ns

FEV_1 and FEV_1/(F)VC are adjusted for age, sex, height, and pack-years. %LAA950 is adjusted for age, sex, pack-years, and weight in both studies, and for center in ICGN. FEV_1/(F)VC = FEV_1/FVC or FEV_1/VC ratios; PI = protease inhibitor. See Table 1 and 2 legends for expansion of other abbreviations.

[a]Post-BD.

[b]$P \geq .10$ are not shown.

Table 4—*The Association Between PI MZ and COPD-Related Phenotypes, Stratified by Level of Smoking Exposure*

Study	COPD		FEV_1, L[a]		FEV_1/(F)VC[a]		%LAA950[b]	
	OR (95% CI)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
Case-control, all subjects								
Pack-y < 20 (n = 734)	4.6 (1.8-11.5)	.001	-0.45 (0.14)	.001	-0.08 (0.03)	.001	6.4 (1.9)	.001
Pack-y ≥ 20 (n = 935)	0.5 (0.3-1.0)	.045	0.11 (0.11)	ns[c]	-0.003 (0.02)	ns	2.8 (1.7)	.095
ICGN, all subjects								
Pack-y < 20 (n = 460)	1.3 (0.6-2.9)	ns	0.04 (0.17)	ns	-0.03 (0.03)	ns	7.6 (1.3)	< .0001
Pack-y ≥ 20 (n = 2,247)	1.6 (0.9-2.9)	ns	-0.12 (0.10)	ns	-0.04 (0.02)	.026	-1.2 (2.0)	ns

FEV_1 and FEV_1/(F)VC are adjusted for age, sex, height, and pack-years. %LAA950 is adjusted for age, sex, pack-years, and weight in both studies, and for center in ICGN. See Table 1, 2, and 3 legends for expansion of abbreviations.

[a]Post-BD.

[b]Information on %LAA950 was only available for 371 case-control subjects with pack-years < 20, 459 case-control subjects with pack-years > 20, 141 ICGN subjects with pack-years < 20, and 703 ICGN subjects with pack-years > 20.

[c]$P \geq .10$ are not shown.

in the low-exposure group, whereas this was not the case in the group with high exposure (Table 4). On the contrary, PI MZ case subjects seemed to have a lower risk of COPD in the high-exposure group. When adding case-control status as a covariate to the quantitative trait analysis (FEV_1, FEV_1/FVC, and %LAA950), these results were no longer significant in the low-exposure group, suggesting that the results were largely driven by the strong association of PI MZ with COPD in this group (7.9% of the case subjects and 1.7% of the control subjects were PI MZ; $P < .001$). In ICGN, PI MZ was associated with a higher percentage emphysema (%LAA950) among subjects with < 20 pack-years of smoking (β = 7.6%; $P < .0001$). The univariate comparisons of mean %LAA950 between PI MM and PI MZ subjects in both studies are shown in Figure 1.

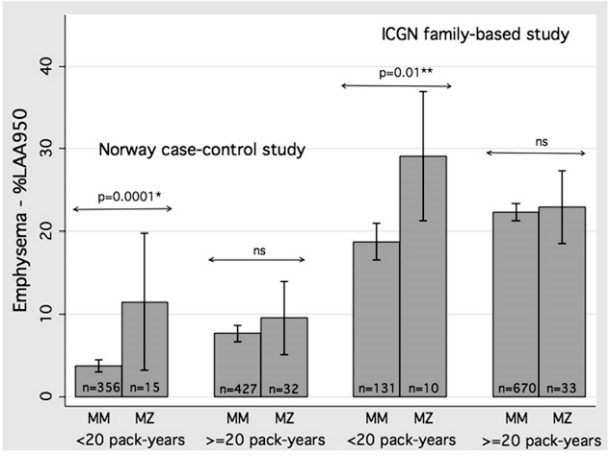

FIGURE 1. Mean percentage of emphysema (%LAA950) in low and high smoking exposure groups by protease inhibitor type. Error bars represent 95% CI for the means. *P value from t test. **P value from random effects mixed-linear model accounting for familial correlations, but without other covariates. ICGN = International COPD Genetics Network; ns = not significant; %LAA950 = percent low attenuation areas < -950 Housfield units.

When testing formally for interactions by including a genotype-by-pack-years interaction term in the regression models, there was a suggestive trend for an interaction for COPD status in the Norway case-control study ($P = .087$). For the other phenotypes, the interaction term was not significant in any population.

DISCUSSION

We examined the associations between AAT PI MZ heterozygosity and COPD-related phenotypes in two large populations: a case-control study from Norway and a multicenter family study from Europe and North America. To our knowledge, this study is the first to examine quantitative CT phenotypes in PI MZ carriers. In both populations, PI MZ was associated with lower FEV_1/(F)VC ratio compared with PI MM. In the case-control study, PI MZ also was associated with more-severe emphysema on chest CT scan.

Our results indicate that PI MZ individuals are slightly more susceptible to the development of airflow obstruction than PI MM individuals, which is consistent with findings in some previous studies but contrary to others. As illustrated by our previous metaanalysis,[6] there have been discrepancies among findings using different study designs. Case-control studies often have found an increased risk of COPD in PI MZ individuals, whereas population-based cross-sectional studies typically have failed to show a difference in lung function between PI MM and PI MZ individuals. Although the metaanalysis supported a small increase in COPD risk, it did not identify a difference in FEV_1 between PI MZ and PI MM individuals. The metaanalysis did not examine FEV_1/FVC ratio or quantitative CT scan measurements.

Several large studies of lung function and PI type have been performed. In a large Danish study of 9,187 individuals from the general population, PI MZ

was associated with reduced lung function in those with clinically established COPD.[15] In another large population-based study of 2,944 subjects, no differences in lung function among PI types were found.[16] The results of published longitudinal studies of decline in lung function over time also have been inconsistent. A general population study in Copenhagen, Denmark, found the annual decrease in FEV_1 to be slightly greater in PI MZ individuals than in PI MM individuals,[17] but another longitudinal study of the general population in Arizona failed to confirm these findings.[18] A 5-year follow-up of smokers in the Lung Health Study demonstrated that rapid decline of FEV_1 was associated with PI MZ, and this association was stronger when PI MZ subjects also had a family history of COPD.[19]

In a family study of individuals with severe AAT deficiency and their relatives, PI MZ relatives of AAT-deficient subjects with COPD had lower FEV_1 than PI MZ relatives of AAT-deficient subjects without COPD.[20] This finding suggests that a subset of PI MZ individuals may have an increased risk for lung disease because of other familial factors and that other genetic or environmental factors may contribute to or modify the risk in PI MZ subjects.

Severe AAT deficiency is associated with early onset of emphysema due to insufficient protection from the action of proteases. This emphysema is most often panlobular and usually shows basal predominance.[21,22] PI MZ heterozygotes have reduced serum levels of AAT (approximately 60% of PI MM levels). In addition, the Z mutation results in a protein that is a less-efficient inhibitor of neutrophil elastase.[23] It is therefore biologically plausible that an increased COPD risk in PI MZ individuals is related to the emphysema component of COPD.

Quantitative measurements of chest CT scan variables were available in a subset of both study populations. Findings in the case-control study suggested more severe emphysema in PI MZ individuals, but these results were not confirmed in ICGN. Because there were few PI MZ individuals with CT scans, small sample size may have contributed to the lack of replication. However, in subjects with relatively low smoking exposure ($<$ 20 pack-years), PI MZ was associated with more-severe emphysema in both populations. No differences were found for airway wall thickness in either study.

Even though PI type is the most important determinant of serum AAT level, it is likely that a combination of several genetic and environmental factors contribute to the disease phenotype in severe AAT deficiency.[24] PI ZZ subjects show significant variability in the development of lung disease,[25] and IL-10 has been identified as a potential genetic modifier for airflow obstruction in severe AAT deficiency.[26] Gene-by-environment interactions also may modify the risk of lung disease in PI MZ carriers.

To investigate whether the effect of PI MZ was modified by level of smoking exposure, we stratified our study populations into low- and high-exposure groups (defined by an arbitrary threshold of 20 pack-years). Individuals who develop COPD at low smoking exposure may have a higher genetic susceptibility and a greater genetic component to their disease than others. Results of the Norway case-control study suggest that the genetic effect of PI MZ heterozygosity may be more important in individuals with low smoking exposure. In ICGN, the same pattern was found for emphysema, but not for the other COPD-related phenotypes. These findings could be an indication of increased genetic risk in the low-exposure population, potentially mediated by a gene-by-smoking interaction. Notably, there was a trend for a genotype-by-smoking interaction for the outcome COPD status in the case-control study. However, when adjusting for case-control status in the Norway case-control study, PI MZ was no longer significantly associated with the quantitative traits (FEV_1, FEV_1/FVC, and %LAA950) in the low-exposure group. Further research is needed to conclude whether the effects of PI MZ heterozygosity differ by level of smoking exposure, preferably using a group of never smokers as reference group. The apparent protective effect of PI MZ in the high-exposure group (OR, 0.5) in the case-control study is not consistent with the rest of our findings. It possibly could reflect that PI MZ individuals quit smoking or smoke less in response to symptoms or disease (the "healthy smoker" effect), but it also could represent a spurious finding.

The limitations of our study should be acknowledged. Our two study samples did not represent population-based samples; therefore, it is uncertain whether our findings apply to the general population. The presented results for the whole case-control study were not adjusted for case-control status. When adjusting for disease status in the case-control study, the results for quantitative traits in the low smoking exposure group (Table 4) were no longer significant, which could relate to the highly significant association of COPD status with PI MZ in the low-exposure group. The importance of adjustment for case-control status in these situations is controversial.[27,28] Even though both the case-control study and the family study had large sample sizes, the number of PI MZ subjects was low, especially for the CT scan phenotype analyses and, thus, may have resulted in low power to detect differences between PI MM and PI MZ groups. The possibility of spurious associations due to population stratification cannot be excluded.[29] Population stratification is unlikely to be a problem in the relatively homogeneous population in the Norway case-control

study but may be relevant in the multicenter ICGN study. Both populations consisted of current and ex-smokers; therefore, we are unable to conclude whether our findings are valid only for PI MZ subjects with a smoking history or whether they can be generalized to all PI MZ subjects.

When comparing PI MZ and PI MM in two independent studies, we found lower FEV_1/(F)VC ratio in PI MZ individuals in both studies. Among subjects with a low smoking history, PI MZ individuals had more severe emphysema on chest CT scan in both studies. It remains uncertain whether all PI MZ individuals have an increased risk or whether a subset is more susceptible because of other genetic or environmental factors. Further studies using well-characterized populations with a larger number of PI MZ individuals, including both smokers and nonsmokers, are likely needed to answer this question definitively.

Acknowledgments

Author contributions: *Dr Sørheim:* contributed to the data analysis and the writing and review of the manuscript.
Dr Bakke: contributed to the data collection and the writing and review of the manuscript.
Dr Gulsvik: contributed to the data collection and the writing and review of the manuscript.
Dr Pillai: contributed to the writing and review of the manuscript.
Dr Johannessen: contributed to the data analysis, statistical support, and the writing and review of the manuscript.
Dr Gaarder: contributed to the protease inhibitor typing in the Norway study and the writing and review of the manuscript.
Dr Campbell: contributed to the data collection and the writing and review of the manuscript.
Dr Agustí: contributed to the data collection and the writing and review of the manuscript.
Dr Calverley: contributed to the data collection and the writing and review of the manuscript.
Dr Donner: contributed to the data collection and the writing and review of the manuscript.
Dr Make: contributed to the data collection and the writing and review of the manuscript.
Dr Rennard: contributed to the data collection and the writing and review of the manuscript.
Dr Vestbo: contributed to the data collection and the writing and review of the manuscript.
Dr Wouters: contributed to the data collection and the writing and review of the manuscript.
Dr Paré: contributed to the data collection and the writing and review of the manuscript.
Dr Levy: contributed to the data collection and the writing and review of the manuscript.
Dr Coxson: contributed to the data collection and the writing and review of the manuscript.
Dr Lomas: contributed to the data collection and the writing and review of the manuscript.
Dr Hersh: contributed to the data analysis, statistical support, and the writing and review of the manuscript.
Dr Silverman: contributed to the data collection, data analysis, statistical support, and the writing and review of the manuscript.
Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following conflicts of interest: Dr Sørheim received lecture fees from AstraZeneca in 2008 and a travel grant and lecture fees from GlaxoSmithKline (GSK) in 2009. Dr Bakke has received all together 1,000 Euros from GSK and 1,000 Euros from AstraZeneca in fees for speaking and is a principal investigator in a GSK-sponsored study. Dr Campbell has a significant financial interest in HerediLab, Inc; has served on and chaired advisory boards and medical educational activities for CSL Behring; and served as a consultant to Talecris Biotherapeutics. Dr Agustí has received research funding and honoraria for speaking engagements and consultant work from GSK, AstraZeneca, Almirall, ALTANA, and Boehringer Ingelheim. Dr Rennard has consulted with, received grants from, or received honoraria for lectures from many companies with interest in COPD and smoking cessation (details available in the full disclosure statement). Dr Vestbo received 276.000 Danish kroner in 1985 in support for a research project from a fund financed by the Scandinavian Tobacco Company. Dr Paré was the principal investigator of a project that was funded by GSK to develop CT scan-based algorithms to quantify emphysema and airway disease in COPD; was the principal investigator of a Merck Frosst-supported research program to investigate gene expression in the lungs of patients with COPD; established a new contract with Merck to discover genetic predictors of gene expression in lung tissue; and sits on an advisory board for Talecris Biotherapeutics, who makes α_1-antitrypsin replacement therapy. Dr Coxson received $4,800 in 2006 and 2007 for serving on the steering committee for the ECLIPSE project for GSK; is the coinvestigator on two multicenter studies sponsored by GSK and has received travel expenses to attend meetings related to the project; and has three contract service agreements with GSK to quantify the CT scans in subjects with COPD and a service agreement with Spiration Inc to measure changes in lung volume in subjects with severe emphysema. A percentage of Dr Coxson's salary between 2003 and 2006 ($15,000/y) derives from contract funds provided to Dr Paré by GSK for the development of validated methods to measure emphysema and airway disease using CT imaging. Dr Coxson is the coinvestigator (D Sin, principal investigator) on a Canadian Institutes of Health-Industry (Wyeth) partnership grant. Dr Lomas has received a research grant and fees for speaking and acts as a consultant for GSK. Dr Silverman has received grant support for two studies of COPD genetics and consulting fees from GSK; has received consulting fees from AstraZeneca; and has received honoraria from Wyeth, Bayer, GSK, and AstraZeneca. Drs Gulsvik, Pillai, Johannessen, Gaarder, Calverley, Donner, Make, Wouters, Levy, and Hersh have reported to *CHEST* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.
Additional information: The e-Appendix can be found in the Online Supplement at http://chestjournal.chestpubs.org/content/138/5/1125/suppl/DC1.

References

1. Laurell CB, Eriksson S. The electrophoretic alpha 1-globulin pattern of serum in alpha 1-antitrypsin deficiency. *Scand J Clin Lab Invest*. 1963;15(2):132-140.
2. Lieberman J, Winter B, Sastre A. Alpha 1-antitrypsin Pi-types in 965 COPD patients. *Chest*. 1986;89(3):370-373.
3. Lomas DA, Parfrey H. Alpha1-antitrypsin deficiency. 4: molecular pathophysiology. *Thorax*. 2004;59(6):529-535.
4. de Serres FJ. Worldwide racial and ethnic distribution of alpha1-antitrypsin deficiency: summary of an analysis of published genetic epidemiologic surveys. *Chest*. 2002;122(5):1818-1829.
5. Brantly ML, Wittes JT, Vogelmeier CF, Hubbard RC, Fells GA, Crystal RG. Use of a highly purified alpha 1-antitrypsin standard to establish ranges for the common normal and deficient alpha 1-antitrypsin phenotypes. *Chest*. 1991;100(3):703-708.
6. Hersh CP, Dahl M, Ly NP, Berkey CS, Nordestgaard BG, Silverman EK. Chronic obstructive pulmonary disease in alpha1-antitrypsin PI MZ heterozygotes: a meta-analysis. *Thorax*. 2004;59(10):843-849.
7. Gulsvik A, Tosteson T, Bakke P, Humerfelt S, Weiss ST, Speizer FE. Expiratory and inspiratory forced vital capacity

and one-second forced volume in asymptomatic never-smokers in Norway. *Clin Physiol*. 2001;21(6):648-660.
8. Patel BD, Coxson HO, Pillai SG, et al; International COPD Genetics Network. Airway wall thickening and emphysema show independent familial aggregation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008; 178(5):500-505.
9. Zhu G, Warren L, Aponte J, et al; International COPD Genetics Network (ICGN) Investigators. The SERPINE2 gene is associated with chronic obstructive pulmonary disease in two large populations. *Am J Respir Crit Care Med*. 2007; 176(2):167-173.
10. Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis*. 1981;123(6): 659-664.
11. Grydeland TB, Dirksen A, Coxson HO, et al. Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking. *Eur Respir J*. 2009;34(4):858-865.
12. Nakano Y, Muro S, Sakai H, et al. Computed tomographic measurements of airway dimensions and emphysema in smokers. Correlation with lung function. *Am J Respir Crit Care Med*. 2000;162(3 pt 1):1102-1108.
13. Coxson HO, Rogers RM, Whittall KP, et al. A quantification of the lung surface area in emphysema using computed tomography. *Am J Respir Crit Care Med*. 1999;159(3): 851-856.
14. Nakano Y, Wong JC, de Jong PA, et al. The prediction of small airway dimensions using computed tomography. *Am J Respir Crit Care Med*. 2005;171(2):142-146.
15. Dahl M, Nordestgaard BG, Lange P, Vestbo J, Tybjaerg-Hansen A. Molecular diagnosis of intermediate and severe alpha(1)-antitrypsin deficiency: MZ individuals with chronic obstructive pulmonary disease may have lower lung function than MM individuals. *Clin Chem*. 2001;47(1):56-62.
16. Morse JO, Lebowitz MD, Knudson RJ, Burrows B. Relation of protease inhibitor phenotypes to obstructive lung diseases in a community. *N Engl J Med*. 1977;296(21):1190-1194.
17. Dahl M, Tybjaerg-Hansen A, Lange P, Vestbo J, Nordestgaard BG. Change in lung function and morbidity from chronic obstructive pulmonary disease in alpha1-antitrypsin MZ heterozygotes: a longitudinal study of the general population. *Ann Intern Med*. 2002;136(4):270-279.
18. Silva GE, Sherrill DL, Guerra S, Barbee RA. A longitudinal study of alpha1-antitrypsin phenotypes and decline in FEV1 in a community population. *Chest*. 2003;123(5):1435-1440.
19. Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Paré PD. Susceptibility genes for rapid decline of lung function in the Lung Health Study. *Am J Respir Crit Care Med*. 2001;163(2):469-473.
20. Silverman EK, Province MA, Rao DC, Pierce JA, Campbell EJ. A family study of the variability of pulmonary function in alpha 1-antitrypsin deficiency. Quantitative phenotypes. *Am Rev Respir Dis*. 1990;142(5):1015-1021.
21. Parr DG, Stoel BC, Stolk J, Stockley RA. Pattern of emphysema distribution in alpha1-antitrypsin deficiency influences lung function impairment. *Am J Respir Crit Care Med*. 2004; 170(11):1172-1178.
22. Shaker SB, Stavngaard T, Stolk J, Stoel B, Dirksen A. Alpha1-antitrypsin deficiency. 7: Computed tomographic imaging in alpha1-antitrypsin deficiency. *Thorax*. 2004;59(11): 986-991.
23. Ogushi F, Fells GA, Hubbard RC, Straus SD, Crystal RG. Z-type alpha 1-antitrypsin is less competent than M1-type alpha 1-antitrypsin as an inhibitor of neutrophil elastase. *J Clin Invest*. 1987;80(5):1366-1374.
24. DeMeo DL, Silverman EK. Alpha1-antitrypsin deficiency. 2: genetic aspects of alpha(1)-antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk. *Thorax*. 2004; 59(3):259-264.
25. Silverman EK, Pierce JA, Province MA, Rao DC, Campbell EJ. Variability of pulmonary function in alpha-1-antitrypsin deficiency: clinical correlates. *Ann Intern Med*. 1989;111(12): 982-991.
26. Demeo DL, Campbell EJ, Barker AF, et al. IL10 polymorphisms are associated with airflow obstruction in severe alpha1-antitrypsin deficiency. *Am J Respir Cell Mol Biol*. 2008;38(1):114-120.
27. Lin DY, Zeng D. Proper analysis of secondary phenotype data in case-control association studies. *Genet Epidemiol*. 2009; 33(3):256-265.
28. Monsees GM, Tamimi RM, Kraft P. Genome-wide association scans for secondary traits using case-control samples. *Genet Epidemiol*. 2009;33(8):717-728.
29. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. *Lancet*. 2003;361(9357):598-604.